Chapter 4

# Mechanisms of Horizontal Gene Transfer

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**Abstract**

Horizontal gene transfer plays important roles in the evolution of *S. aureus*, and indeed, a variety of virulence factors and antibiotic resistance genes are embedded in a series of mobile genetic elements. In this chapter, we review the mechanisms of horizontal gene transfer, including recent findings on the natural genetic competence. Then, we consider the transfer of two important antibiotic resistance genes: the methicillin resistance gene, *mecA* (in Staphylococcal Cassette Chromosome) and the linezolid resistance gene, *cfr* (in plasmid). In either case, distinct mechanisms driving the gene dissemination support the prominent evolutionary ability of this important human pathogen.

**Keywords:** Transduction, Conjugation, Transformation, staphylococcal cassette chromosome (SCC), *cfr*

## 1. Introduction

*Staphylococcus aureus* is an opportunistic pathogen responsible for diverse infectious diseases ranging from food poisoning and superficial skin abscesses to more serious infections such as pneumonia, meningitis, osteomyelitis, septicemia, endocarditis and toxic shock syndrome. The resistance to a wide variety of antibiotics [1, 2] is a global concern in clinical settings, and methicillin-resistant strains (MRSA), the most common cause of nosocomial infections, are now spreading into the community [3]. The emergence and dissemination of further resistant strains such as vancomycin-resistant *S. aureus* (VRSA) [4] and linezolid-resistant *S. aureus* (LRSA) [5, 6] are anticipated. Major parts of staphylococcal virulence and antibiotics resistance are acquired characteristics, evidenced by many of the toxin and antibiotic resistance genes embedded in mobile genetic elements such as transposons, bacteriophages, insertion

sequences, pathogenicity islands and the staphylococcal cassette chromosome (SCC) [7, 8]. In this chapter, we review the current understanding about horizontal gene transfer (HGT) in *S. aureus* including the recently discovered natural transformation. We also discuss how two important mobile genetic elements (SCC and *cfr* plasmid) would be transferred from cell to cell.

## 2. Horizontal gene transfer mechanisms

### 2.1. Phage-related mechanisms

Phage-mediated horizontal gene transfer is the major driving force for *S. aureus* evolution and is well reviewed elsewhere [9]. The experimental protocols for the phage transduction are also established [10]. Staphylococcal phages can also serve as a helper phage to transfer *Staphylococcus aureus* pathogenicity islands (SaPIs) [11]. SaPI carries toxin genes including the toxic shock syndrome toxin 1 and superantigens. In addition to the conventional transduction by staphylococcal phages, atypical giant phage in environment is also capable of transduction [12].

In 1970s, a transformation-like phenomenon (now termed "pseudo-competence" or "pseudo-transformation") was described [13]. A series of studies have confirmed that it is a HGT mechanism that requires the presence of a staphylococcal phage [14]. The "competence-conferring factor" was most likely the phage tail that has lytic activity. In some old bacteriology books, pseudo-competence is regarded as competence, but the first report on genuine natural genetic competence was published on 2012 [15]. Pseudo-competence was demonstrated to be distinct from natural competence: the important competence genes encoded in the *comG* and *comE* operons were dispensable for pseudo-competence [15].

### 2.2. Conjugation

Bacterial conjugation has been studied in Gram-negative and Gram-positive species. Although broad-host-range plasmids able to replicate in both groups exist, the differences in terms of membrane and peptidoglycan cell wall require different conjugation systems on the basis of cell-to-cell recognition and contact.

Most of the conjugative staphylococcal plasmids studied belong to the incQ family. One of the better known staphylococcal conjugative plasmid is pGO1 [16], considered as the prototype of this type of plasmids. All the conjugative genes are located on a 14.5 kb region, and the minimal machinery necessary for conjugation includes the *oriT*, a nickase protein (*nes*) and the *tra* operon. This plasmid shows high similarities, in terms of genetic organization of the *tra* operon, with other Gram-positive conjugative plasmids such as the staphylococcal pSK41 plasmid [17], the lactococcal pMRC01 [18] and the enterococcal pRE25 [19].

Staphylococcal plasmids related to the pGO1/pSK41 family share an important homology regarding the organization of conjugative genes and, in addition, present an identical IncQ-type

relaxase and a nickase gene (*nes*) responsible for the generation of the nick at the *oriT* [17]. The plasmids belonging to this family are self-conjugative, and they are able to mobilize small non-conjugative coresident plasmids. They are also associated with gentamicin (and other aminoglycosides) resistance and can be related to resistance to penicillin, trimethoprim, bleomycin, tetracycline, macrolides, lincosamide, streptogramin B and antiseptics [20]. These plasmids have contributed to the evolution of staphylococcal species in antibiotic-enriched environments, and recently, they have been reported to be related to resistance against the most important antimicrobials used in MRSA treatment: linezolid and vancomycin.

### 2.3. Natural transformation

Natural transformation requires the uptake of environmental DNA by the action of a set of DNA-uptake proteins that are expressed in the bacterial membrane. Once DNA is incorporated into the cytoplasm, it can be used as a source of nutrients, as a template to repair damaged genetic material or to enhance bacterial fitness by generating diversity or introducing novel traits [21].

To undergo transformation, bacteria need to develop a specific physiological state called genetic competence. Competence is achieved through the regulated expression of the genes encoding the DNA uptake machinery [22]. In general, Gram-positive DNA uptake machinery is formed by a pseudopilus (ComG proteins) that brings extracellular DNA to the cytoplasmic transport machinery, a DNA-binding protein (the receptor ComEA) and a channel (ComEC). Only a single strand enters the cytosol, while the complementary strand is degraded by an endonuclease [23].

*S. aureus* had been regarded as a non-transformable species until natural transformation was demonstrated in 2012 [15]. Natural transformation can transfer long DNA fragments that are too large to be transferred by bacteriophages (transduction) [22]. Indeed, the long staphylococcal cassette chromosome *mec* (SCC*mec*) type II element was successfully transferred by transformation [15], leading to the idea that the exchange of large DNA regions between distinct *S. aureus* clonal complexes may be also due to the natural transformation [24].

The regulation of competence development is a species-specific process. In *S. aureus*, the main competence regulator is the alternative sigma factor H (SigH). SigH activates the transcription of the competence machinery genes (*comG* and *comE* operons) that are essential for the development of natural transformation [15, 25]. In addition, the transcription factor ComK enhances the expression of the SigH regulon [26]. SigH is expressed in a minor population by two distinct mechanisms. The *sigH* gene can be rearranged by a "short-junction duplication," or be post-transcriptionally regulated through an inverted repeat (IR) sequence at the 5′-UTR of *sigH* mRNA. The IR is thought to hinder the ribosome-binding site to suppress the SigH expression, but the activation mechanism is not clarified yet.

Competence development is a species-specific process that requires particular environmental conditions. These conditions include nutrient access, starvation, altered growth conditions and cell density [22]. Natural transformation in *S. aureus* is detectable under specific conditions

when cells are grown in the chemically defined CS2 medium [15]. Under these growth conditions, wild-type strains (N315 and its derivative) show low transformation frequencies ($<10^{-11}$), partly attributed to the subpopulation limited SigH expression. Overexpression of SigH increases the transformation frequencies up to $\sim10^{-9}$ when purified plasmid DNA is used as donor and to $\sim10^{-7}$ if living *S. aureus* COL cells carrying pT181 are used. Moreover, there seems to be more preferable growth conditions that facilitate transformation (Ohniwa et al., in preparation). So far, we achieved c.a. $10^{-6}$ level frequency in the unmodified N315 derivative strains, as well as in a part of the clinical isolates, which will be published elsewhere.

Even in SigH-expressing cells, the transformation frequencies change depending on the growth conditions, suggesting that there are additional levels of regulations for an efficient transformation. Importantly, antimicrobial agents also affect the transformation efficiencies in the SigH-expressing cells [27]. **Table 1** summarizes the effect of the antibiotics in *S. aureus* and other species. **Table 1** also includes the SOS response, because it is a complementary response in some bacteria: antibiotics that induce SOS response, such as fluoroquinolone or mitomycin C, induce competence in species lacking the SOS system [28–30] but suppress competence in species harboring the SOS system [31]. Although SOS response in *S. aureus* is limited and its accessories are simple, it does exist [32, 33]. The treatment with mitomycin C suppresses transformation in *S. aureus*. However, ciprofloxacin (fluoroquinolone) has no effect. This might indicate that the interplay between natural transformation and SOS response cannot be simplified. *S. aureus* response to β-lactam antibiotics is also linked to SOS response [34–36]. Fosfomycin and vancomycin increase natural transformation in SigH-expressing cells, but the detailed response mechanism involved is not known. Whether these inducing effects can be observed in the unmodified strains needs to be tested in a future study.

| | | *S. aureus* **(+ SigH cells)** | | *S. thermophilus* | | **S. p*** | **L. p*** | **H. p*** |
|---|---|---|---|---|---|---|---|---|
| | | **TF**** | **SOS** | **TF**** | **SOS** | **TF**** | **TF**** | **TF**** |
| | Fosfomycin | + [27] | | | | | | |
| | Vancomycin | +[27] | | | | No effect [28] | | |
| | Oxacillin | - [27] | Yes [34, 35] | | | | | |
| | Cefazolin | - [27] | | | | | | |
| | Ampicillin | | Yes [36] | | | No effect [28] | No effect [29] | No effect [30] |
| Quinolones | Ciprofloxacin | No effect [27] | Yes [32] [37] | | | | | + [30] |
| | Norfloxacin | | | - [31] | Yes [31] | + [28] | + [29] | |
| | Mitomycin C | - [27] | Yes [37] [38] | - [31] | Yes [31] | + [28] | | |

*S. p, L. p, H. p lack SOS system [29, 33].
**TF: transformation and/or competence gene expression.
S. p: *Streptococcus pneumoniae*, L. p: *Legionella pneumophila*, H. p: *Helicobacter pylori.*

**Table 1.** Effects of antibiotics on transformation and SOS response.

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## 3. Dissemination of antibiotic resistance determinants

Since Fleming's discovery of penicillin and its application to treatment, *S. aureus* has experienced a variety of antibiotics in clinical settings. Penicillin-resistant *S. aureus* was described before the introduction of penicillin to the market in 1943. It carried a β-lactamase gene in a plasmid. Methicillin was developed as a β-lactam derivative that cannot be degraded by β-lactamase, but methicillin-resistant *S. aureus* (MRSA) was described soon after its introduction. The methicillin resistance gene is in a mobile genetic element designated staphylococcal cassette chromosome (SCC). Vancomycin is one of the effective resources for MRSA treatment, though vancomycin-resistant *S. aureus* (VRSA) has already been reported [39]. Linezolid is another promising anti-MRSA drug, but the dissemination of linezolid resistance is also anticipated. The antibiotic resistance genes and a series of reports on their transfer mechanisms are summarized in **Table 2**. Here, we focus on the dissemination mechanisms of SCC and linezolid resistance.

### 3.1. Dissemination of SCC

β-lactams were the first line of antibiotics against *S. aureus* infections. However, resistance has rapidly emerged. The first methicillin-resistant *S. aureus* (MRSA) strain was identified in 1961, only one year after the clinical introduction of methicillin [76]. Methicillin is a semisynthetic penicillin that resists the action of β-lactamases. It was introduced as a first choice treatment against infections caused by penicillin-resistant *S. aureus*. The methicillin resistance gene (*mecA*) encodes an alternative penicillin-binding protein (PBP2a) that has low affinity for all β-lactams. PBP2a can maintain the cell wall synthesis, while all the other PBPs are inhibited by β-lactams [77].

The *mecA* gene is carried in a variable mobile element called staphylococcal cassette chromosome *mec* (SCC*mec*). SCC*mec* is integrated in a unique orientation into a specific chromosomal attachment site (*attB*SCC*)* [78]. The basic structure of the SCC*mec* element consists of a cassette chromosome recombinase (*ccr*) and a *mec* complex. The *mec* complex contains the *mecA* gene and its regulatory genes (not always present) [79]. The cassette is classified according to a combination of both complexes [80]. The International Working Group on the Staphylococcal Cassette Chromosome elements reports 11 types of *SCCmec* (**http://www.sccmec.org/Pages/SCC_TypesEN.html**). SCC*mec* varies in size (from 20 to 67 kb), and it can carry other elements such as resistance genes, insertion elements, plasmids or transposons. SCC*mec* elements have only been found in staphylococci with the exception of *Macrococcus caseolyticus* [43]. This species is closely related to the staphylococci, and it was part of the *Staphylococcus* genus until 1998 when it was reclassified [81].

MRSA strains appeared in the hospital environment and spread rapidly causing serious clinical problems and several hospital outbreaks. The first MRSA strain was identified in the United Kingdom in 1961, and it carried the type I SCC*mec.* Types II and III were identified in the early 1980s in Japan and New Zealand, respectively. These types of SCC*mec* were all identified in the hospital environment; they are the largest types and can carry additional

| Type | Antibiotic | Gene | Location | Origin | Reported/probable HGT mechanism | Refs. |
|---|---|---|---|---|---|---|
| β-lactams | Penicillin | *blaZ* | Plasmid (transposon) | *S. haemolyticus?* | Conjugation Pseudo-transformation | [40–42] |
| | Methicillin | *mecA* | Chromosome (SCC*mec*) | CoNS | Transduction Conjugation Transformation | [15, 43–49] |
| Glycopeptides | Vancomycin | *vanA* | Plasmid (transposon) | *Enterococcus* spp. | Conjugation | [50, 51] |
| Aminoglycosides | Gentamicin Kanamycin Tobramycin | *aacA–aphD* | Plasmid (transposon) | *Bacillus* spp.? | Conjugation Transduction | [52] |
| Antifolates | Trimethoprim | *dfrA* | Plasmid (transposon) | *Bacillus* spp.? | Conjugation | [53, 54] |
| | | *dfrG* | Chromosome (IS) | *E. faecium?* | – | [55] |
| | | *dfrK* | Plasmid Chromosome (transposon) | ? | Conjugation - | [56–58] |
| Macrolide Lincosamide Streptogramin B | | *ermB* | Plasmid (transposon) | Streptococci | Conjugation Transduction | [59–61] |
| | | *ermC* | Plasmid | CoNS | Transduction | [60, 62] |
| | | *ermA* | Chromosome (transposon) | CoNS | Conjugation | [60, 62, 63] |
| Tetracyclines | Tetracycline | *tetK tetL* | Plasmid | Streptococci Enterococci | Conjugation | [64, 65] |
| | | *tetM* | Chromosome (transposon) | Streptococci *E. faecalis* | Conjugation | [61, 64] |
| Chloramphenicol | Chloramphenicol | *cat* | Plasmid | *S. epidermidis* | Conjugation Transduction | [8, 66, 67] |
| Oxazolidinones | Lynezolid | *cfr* | Plasmid | CoNS? | Conjugation Transduction | [6] |
| Streptogramins | Dalfopristin | *vgaA, vgaB, vatA, vatB, vatC* | Plasmid | ? | Conjugation | [68, 69] |
| Fusidanes | Fusidic acid | *fusB* | Chromosome (SaPI) Plasmid | CoNS | Transduction | [11, 70] |
| | | *fusC* | Chromosome (SCC) | CoNS | – | [7 –75] |
| Phosphonic acids | Fosfomycin | *fosB* | Chromosome (SaPI) | ? | Transduction | [11] |

**Table 2.** Antibiotics resistance genes.

resistance elements; strains that carry them are usually multi-resistant [78, 82]. During the 1990s, community-associated MRSA strains were identified, and during the early 2000s, the SCC*mec* type IV and V were described in USA and Australia, respectively. These types are smaller elements that rarely carry extra resistance elements, and they are described as community-associated SCC*mec* types [83, 84]. Types I to V are the main and widely distributed elements; since their description, new variants have been reported (SCC*mec* VI–XI).

The origin of the SCC*mec* element is not clear, but evidence indicates that it comes from coagulase negative staphylococci (CoNS). Ancestral forms have been identified in *S. sciuri, S. fleuretti, S. xylosus, S. hominis* and *M. caseolyticus* [43–45]. The source of the *mec* and *ccr* complex is unknown, but they may have assembled into *SCCmec* in CoNS where they were modified and then transferred to *S. aureus* [46]. The primary source of SCC*mec* for *S. aureus* seems to be *S. epidermidis.* SCC*mec* type IV shares high homology between both species [85]; *S. haemolyticus* and *S. hominis* appear to be reservoirs for specific classes of *mec* complex and *ccr* genes [86, 87].

The transfer mechanism of SCC*mec* is not well defined. Successful transfer via transduction, conjugation and natural transformation has been reported. Although transduction of small SCC*mec* elements (type IV, I and V) has been described [47, 48], it is unlikely that this is the predominant method. Many of the SCC*mec* are too big (up to ~60 kb) to be encapsulated by a phage. Conjugation is an alternative mechanism. Ray et al. demonstrated the transfer of a 30.8 kb element (modified from SCC*mec* II) via conjugation, the spontaneous excision from the conjugative plasmid and the insertion into the recipient chromosome of *S. aureus* and *S. epidermidis* [49]. However, these authors induced the transfer by overproducing *ccrAB.* Natural transformation can also explain the transfer of large SCC*mec* types. We have successfully transferred the SCC*mec* II [15]. However, the interspecies transfer by natural transformation has not been tested.

### 3.2. Dissemination of *cfr*

The *cfr* gene was identified in 2000; it was described as a new chloramphenicol/florfenicol resistance element located on the pSCFS1 plasmid [88]. This plasmid was the first multi-resistance plasmid found in a *Staphylococcus sciuri* (isolate from the nasal swab of a calf) and was associated with resistance to chloramphenicol, florfenicol, spectinomycin and MLSB (macrolide, lincosamide and streptogramin B) antibiotics. The *cfr* gene was associated with the tn558 transposon, closely related to the tn554 associated with erythromycin resistance [89].

The *cfr* gene was not associated with clinical cases until 2007, when it was demonstrated that this gene was responsible for the elevated MIC to linezolid in one clinical MRSA strain isolated in Medellin (Colombia) [90]. Linezolid is a synthetic inhibitor of protein synthesis. Its activity against Gram-positive bacteria (including MRSA and *Enterococcus* sp., even in the case of reduced vancomycin susceptibility) made this antibiotic an essential tool in the treatment of resistant pathogens [91].

Up to 2007, the only known mechanism for linezolid resistance known in staphylococci was the spontaneous mutations in ribosomal proteins [92]. This non-transmissible mechanism

was associated with the previous intensive use of linezolid. The association of a potential transmissible mechanism of resistance to this antibiotic represented a global concern due to the scarce alternatives for the infections caused by these pathogens and also, due to the potential spreading of this resistance mechanism to the pathogenic bacterial pool.

When the first *cfr*-positive clinical isolate was detected, sequencing analysis showed the absence of any mutation previously associated with linezolid resistance. The strains showed an unexpected post-transcriptional modification at A2503 in the 23S rRNA. The mechanism underlying the resistance conferred by *cfr* is related to the modification of the antibiotic target site on the 23S ribosomal RNA [90]. Thus, cfr is a methyltransferase that causes a reduced ribose methylation at C2498 and the addition of an extra methylation at A2503.

In 2008, the first outbreak of linezolid-resistant MRSA strain was reported in Spain [93]. The outbreak took place in the intensive care unit (ICU) of a public hospital and lasted 3 months. A total of 15 patients infected or colonized with linezolid-resistant MRSA were detected. In this case, some isolates showed identical PFGE profiles, showing the clonal dissemination of the same linezolid-resistant strain, but other *cfr*-positive strains showed a different PFGE profile. As *cfr* prevalence was extremely low, the existence of a horizontal gene transfer event was strongly suggested, and specially, the potential existence of some undetected reservoir, capable of spreading the *cfr* gene among the pathogenic staphylococcal pool, was hypothesized. The association of a potentially transmissible mechanism of resistance to this antibiotic represented a global concern due to the scarce treatment alternatives and the potential spreading to the pathogenic bacterial pool.

In 2008, the presence of plasmid-borne *cfr* in two strains isolated in Ohio hospitals was described [94]. In this case, two staphylococci, one MRSA and one *S. epidermidis*, with linezolid MICs of 8 and 256 mg/L, respectively, were isolated from two different patients. Sequence analysis found two different plasmids on the basis of the *cfr* insertion context. The *S. epidermidis* insertion couldn't be determined, but the plasmid harbored by the *S. aureus* strain showed a pSCFS3-like genetic environment [95]. In this genetic context, the Tn558 transposon was truncated by the tandem insertion of *istAS-istBS*, potentially related to *cfr* gene mobilization. Since 2008 several studies detected the presence of this gene in livestock-associated strains belonging to different bacterial species such as *Proteus vulgaris*, *Enterococcus* spp., *Macrococcus caseolyticus* or *Jeotgalicoccus pinnipedialis* [96–98]. These findings showed the spread of these resistance traits among livestock-associated bacteria known as reservoir for clinical-associated strains.

In 2010, during the analysis of a collection of Panton-Valentine leukocidin (PVL)-positive MRSA isolates from Ireland, one *cfr*-positive strain was detected [99]. This strain harbored a ca. 40 kbp plasmid with *cfr* associated with the Tn558 transposon. However, this gene was located in a genetic context not previously described. In this plasmid, named pSCFS7, *cfr* gene was found inserted in the *tnpB* gene reading frame. In this case, this gene was truncated by the insertion of the terminal region of *istBS*. The detection of the pSCFS7 plasmid was especially relevant; on one hand, the *cfr*-positive MRSA strain belonged to the USA 300 genotype (ST8-MRSA-IVa), which is predominant among community-acquired MRSA

(CA-MRSA) in the United States [100]. And on the other hand, pSCFS7 plasmid demonstrated the capability to be transferred by conjugation to other *S. aureus* strains. Thus, this was the first report of a conjugative plasmid on *S. aureus* strain belonging to genotypes with clinical relevance.

Linezolid susceptibility among clinically significant isolates is monitored by different surveillance programs, such as Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) and the USA Linezolid Experience and Accurate Determination of Resistance (LEADER). According to the results obtained by these programs, linezolid resistance was 0.05% for *S. aureus* and 1.4% for CoNS between 2002 and 2010 [101, 102]. CoNS, not considered as true pathogens, show higher levels of resistance and could act as *cfr* reservoir for the *S. aureus* pool. In fact, the high incidence of unique clones (40%) among *cfr*-positive MRSA suggested that the transmission of the *cfr* gene by HGT could be a common phenomenon [5].

The impact of the transmission of *cfr* among potential reservoirs was determined in Spain after the *cfr*-positive MRSA outbreak in the same hospital [103]. In this study, 100 linezolid-resistant *S. epidermidis* strains obtained between 2008 and 2011 were analyzed. Authors did not recover *cfr*-positive MRSA strains, but they detected this gene in the 58% of the linezolid-resistant *S. epidermidis* isolates, again suggesting the potential role of CoNS as linezolid resistance reservoirs. In 2012, two geographically independent staphylococci harboring conjugative *cfr*-associated plasmids were detected in Spain. One of them was located on a MRSA belonging to ST125 genotype, prevalent among hospital-associated strains (HA-MRSA). This plasmid, named pERGB, showed a new genetic environment of *cfr* insertion, associated with the *istAS-istBS* tandem but not with the TN558 transposon [104]. In addition, it also showed the ability to be transferred by conjugation to the ATCC 29213 *S. aureus* strain.

The second *cfr*-associated vector detected in Spain was found on a ST22 *S. epidermidis* strain [105]. In this case, *cfr* was inserted on a genetic environment identical to the pSCFS7 vector and the plasmid also showed an *in vitro* conjugative transmission. This was the first report of pSCFS7-like plasmids in Spain associated with clinical staphylococci, followed by the finding of two more strains harboring similar vectors in 2014 [106]. Two staphylococcal strains (one *Staphylococcus haemolyticus* and one *S. aureus*) obtained from two clinical cases of septic shock were identified in the same hospital in Spain. Both strains harbored similar ca.40 kb conjugative pSCFS7-like vectors. Although plasmid restriction analysis profiles showed small differences between both strains, the emergence of unrelated *cfr*-positive *S. aureus* and CoNS and the presence of this gene in similar pSCFS7-like plasmids in Spain [105, 106] suggest the potential spread of these vectors among the staphylococcal pool in Spain.

While the observed situation suggested the spread of pSCFS7 among the staphylococci in Spain, in the USA, the situation regarding the prevalence of *cfr* vectors was different. In addition to the pSCFS3-like vector described in 2008 [94], *cfr*-positive strains carrying these plasmids were found in 2013 [107]. The study comprised 19 *S. epidermidis* and 2 *S. aureus* linezolid-resistant strains. Among the studied strains, one *S. aureus* strain did not share a similar plasmid profile. Plasmid sequence analysis demonstrated the existence of identical 39.3 kb

pSCFS3-like plasmids in one *S. epidermidis* and *S. aureus* isolates [6]. As pSCFS3 plasmids were considered as non-conjugative vectors [94], this indirect demonstration of plasmid transmission questioned the nature of the HGT involved in the spread of these plasmids. In addition, an unexpected result was obtained analyzing the sequence of two *cfr* plasmids obtained in staphylococcal clinical isolates from German hospitals [108]. In this study, 6 *cfr*-positive strains were identified among 36 linezolid-resistant *S. epidermidis* isolated between January 2012 and April 2013. Sequence analysis showed the existence of pSCFS6 and pSCFS7-like plasmids. The pSCFS6-like plasmid showed substantial homology to pGO1 plasmid, meanwhile the pSCFS7, as well as pSCFS3-like plasmids, showed the most significant homology to pSK73 plasmid [107]. The presence of pSCFS7-like plasmids in clinical isolates from Germany may suggest the potential spread of these plasmids among European hospitals. Nevertheless, although pSCFS6 harbored the pGO1 *tra* and *nes* conjugative machinery, none of these genes were located in the pSCFS7 sequence, suggesting potential mobilization events of *cfr* environment onto different plasmids backbones [108].

Although conjugation alone was the recognized transmission mechanism for the *cfr* gene, the presence of identical putative non-conjugative pSCFS3 plasmids in different staphylococcal species suggested the existence of other HTG mechanisms involved in the spreading of linezolid resistance [107]. Our group answered this question demonstrating an alternative mechanism for *cfr* spread based on phage transduction among MRSA [6]. By using a *S. aureus* N315 derivate strain harboring a pSCFS7-like vector obtained by conjugation from one clinical *S. epidermidis* strain, we transferred this gene to other MRSA strains by conjugation as well as transduction. In addition, this transmission allowed transductant MRSA *cfr*-positive strains to retain the conjugative capability, suggesting the complete transmission of this vector, or at least all the necessary genes to allow conjugative transmission.

## 4. Conclusion

The prominent evolutionary ability of *Staphylococcus aureus* partly relies on the gene transfer mechanisms ranging from the conventional phage transduction and conjugation to the unique staphylococcal mechanisms such as SaPI-helper phage. Recently found staphylococcal natural transformation further explains the ability to transfer larger genetic elements. The surveillance for antibiotics resistance (especially for the last resort antibiotics such as linezolid and vancomycin) is critical, and the test of emerging resistant pathogens in terms of their ability to use these distinct gene dissemination pathways might help to control the evolution of this important human pathogen.

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